

PROPAGATING *DIONAEA* BY TISSUE CULTURE USING FLOWER STALKS

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Introduction

It was inevitable. As a novice carnivorous plant enthusiast in 2002, I had been scouring the internet educating myself about carnivorous plants and how to grow them, and I encountered numerous references to tissue culture as a means of propagating plants. The advantages of tissue culture are the ability to rapidly propagate plants, and for cloning cultivars or endangered plants which can not be propagated efficiently by any other method.

This intrigued me because I was spending a lot of money increasing my modest collection, and I wondered if it would be possible to propagate carnivorous plants this way. Planting seed, making divisions or leaf pullings (a very common way to propagate *Dionaea*) worked, but was slow. I discovered the "Kitchen Culture Kit" website (<http://www.kitchenculturekit.com>), and decided to try it on my carnivorous plants. My first cultures were *Dionaea* 'Dentate Traps' and *Dionaea* 'Red Dragon' cultivars. Both cultures were successful, with over 25 plantlets of each type being produced from only a single leaf trap cutting from each plant in only a matter of months! Despite the perception that tissue culture is somewhat difficult, and requires an expensive lab environment and equipment, it can actually be done at home with simple equipment for relatively little money (see Figure 1). The "Kitchen Culture Kit" costs less than \$100 (US), and includes media, hormones, baby food jar caps, measuring spoons, droppers, pH test papers and instructions with a Material Safety Data Sheet CD.



Figure 1: Tissue culture tools and supplies.

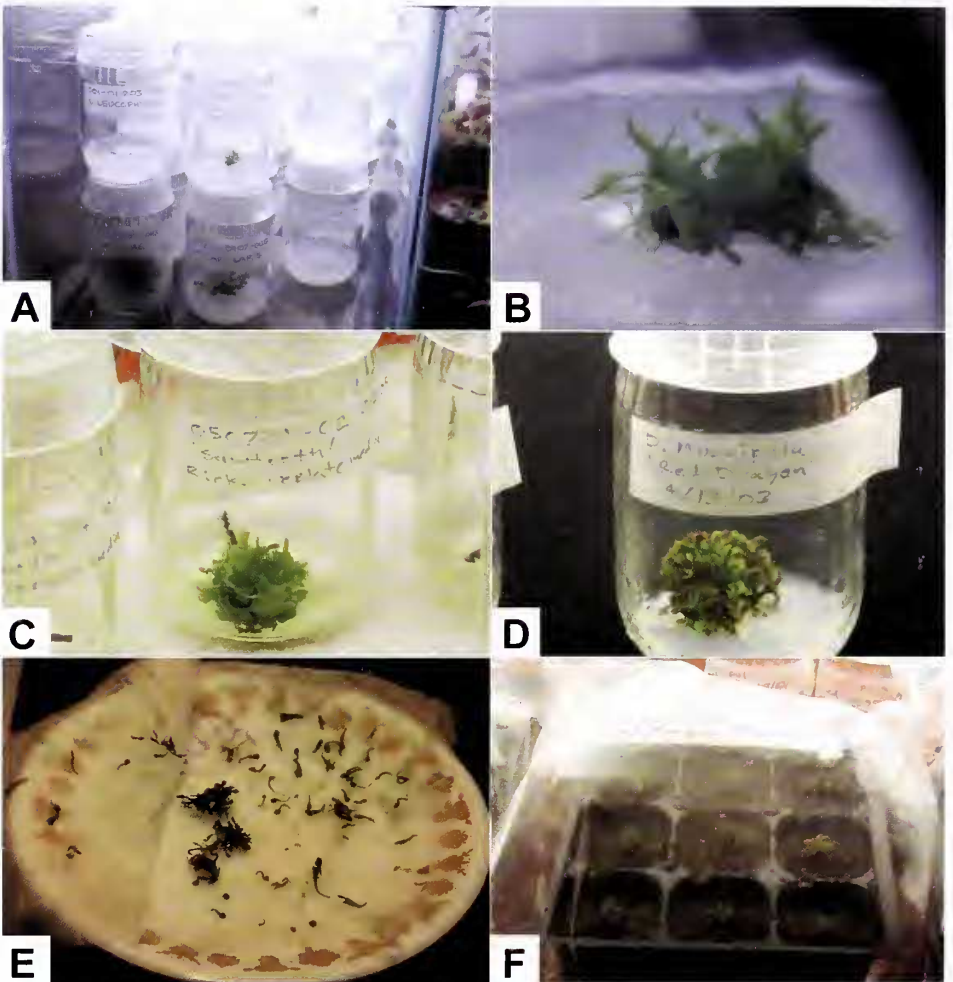


Figure 2: A: Home tissue culture of *D. muscipula* 'Dentate Traps' after being started three months from a single leaf trap. B)Close view of growth at three months. C)*Dionaea* 'Sawtooth' culture at approximately two months. D)*Dionaea* 'Red Dragon' at five months, just before transplanting. E)Look at all the *Dionaea* 'Red Dragon' plantlets! Each clump contains approximately 30 plantlets with leaves and roots. F)*Dionaea* 'Red Dragon' hardening off.

In tissue culture terminology, plant tissue material taken from a plant is called an “explant.” Around a dozen or more plantlets can be produced from a single explant. The resulting plantlets can either be transplanted or sub cultured to produce more plantlets, which over the course of a year could conceivably multiply into the thousands.

Every summer, growers cut off and discard emerging *Dionaea* flower stalks in order to conserve the plants’ energy. I decided to try using the flower stalks as explant material for tissue culture. I did not know if it would work, because to my knowledge, no one has documented propagating *Dionaea* by tissue culture using flower stalk cutting material. However, I have confirmed that it can and does indeed work (see Figure 2)!

I have found that when using *Dionaea* flower stalks as explant material, you can use any part of the flower stalk—from the base, all the way to the top of the inflorescence. The portion at the top (where the flowers are) can be used, however, sterilizing this complicated structure sufficiently is difficult and is likely to be unsuccessful in many cases. The age of the inflorescence



Figure 3: A simple cleanbox or transfer hood.



Figure 4: *Dionaea* 'Sawtooth' flower stalk culture at seven weeks. The inset shows the emerging new growth.

does not seem to be very important. I have used very young flower stalks, and flower stalks that were just about ready to flower. The flower stalks do not even have to be very fresh; I have been sent flower stalk material (packed in small plastic baggies containing medium), which were put into medium days later, and was successful in culturing plants!

Process and Procedures

The process is relatively simple. You must first prepare all the materials you will need, such as sterile capped containers containing a gelled medium solution. For the gelled medium, I have been using the formula listed in Rick Walker's on-line article (Walker 1996). The medium consists of the following: 1/2 strength MS Salts (Murashige and Skoog medium), 100mg/l Casein, 100mg/l inositol, 0.2 mg/l NAA and 5.0 mg/l 2iP, 30000 mg/l sucrose and 7 g/l agar. Casein and Inositol can sometimes be found at health or natural food stores. I normally use one packet of MS to make two liters of medium at a time. For more information on these components, refer to Darnowski (2004).

I take 150ml of the medium, add the sugar, and then adjust the pH to around 5.9 using either baking soda to increase the pH, or vinegar to decrease the pH. I use a small plastic syringe (from a farm feed store) to dispense the medium into six baby food jars (25ml per jar). I also add a small amount of agar (as a gelling agent) to each jar, and then cover them with polypropylene-magenta "B-Caps" (caps made specially to fit the kind of baby food jars I use), or metal lids punched with small holes which are covered with waterproof adhesive bandages. The jars are sterilized in a pressure cooker for 15 minutes. I wrap all my cutting and manipulation tools (such as tweezers and knives) in aluminum foil and sterilize them at the same time.

When the jars cool, they are moved into a transfer hood (see Figure 3). This can be any sort of open boxy container that serves to help minimize flow of airborne contaminants such as dust, bacteria and mold. Mine is simply a plastic storage container turned on its side, with a piece of transparent film taped over the front. It is a good idea to wipe out the interior of the box with alcohol, and even spray alcohol into the air adjacent to the box to help settle any airborne contaminants. Cleanliness during culturing operations is very important! Wash hands thoroughly (I also spray them with alcohol), or wear surgical gloves. Minimize any air movement in the vicinity by closing doors and windows, and move about the area slowly. I use a spray bottle of isopropyl alcohol to resterilize my hands and tools used in the transfer hood.

Next, cut a *Dionaea* flower stalk into pieces a bit shorter than the width of the mouth of the jars holding the medium. Move these explants into the transfer hood, and surface-sterilize them in separate solutions of 70% isopropyl alcohol, 10% bleach, and 3% hydrogen peroxide for perhaps 1-3 minutes each. Rinse the explants in sterile water and place them onto the surface of the medium in each baby food jar. The explants must be in direct contact with the medium—it does not seem to matter if they are on the surface, partially submerged, or completely submerged. Recap the jars and seal them as I described above. The jar and lid can be sealed using a plastic film tape if desired, but in most cases I have not found it necessary. Perform the transfers as quickly as possible to reduce the possibility of airborne contaminants entering the jars.

When all jars have been prepared and sealed, label them and transfer them to a shelf approximately 25-30 cm (10-12 inches) below fluorescent lights. My lights are operated by a timer set to provide around 18 hours of light per day. Visually check the jars periodically for signs of contamination. If contamination occurs, the contents of the jar should be discarded. If the explant is valuable and still alive, you can try to resterilize it and return it to a new jar of medium (all this should be done in the transfer hood, of course).

With luck, in perhaps 4-8 weeks the explants should swell and new growth should start (see Figure 4). The new growth will usually consist of a tightly packed mass of plantlets with leaves (fully formed with traps) and roots. However, some varieties grow just a few large plantlets. The plantlet mass can be removed from the jar, divided, and either transplanted to soil, or sub-cultured into fresh jars of media to be multiplied even further.

While propagating *Dionaea* from flower stalk material does work, problems can and likely will be encountered. The three main problems I have experienced are mold or bacterial contamination, phenolic bleeding, and callus formation. Symptoms of mold and bacterial contamination are obvious—the contaminants can be seen growing on the explant or into the medium. This is a result of either insufficient sterilization or poor procedures. If you have problems with this, review your techniques, talk to other tissue culture practitioners, and try again. Phenolic bleeding occurs as a result of the plant material attempting to deal with the affects of a cut wound. Generally the symptom will be a dark reddish-violet or intense yellow color that spreads throughout the media. The yellow phenolics are so volatile they will usually stain the plastic caps! Resterilization and reculturing may reduce and eliminate the bleeding. Callus formation is the result of rapid, undifferentiated cell growth resulting in an amorphous lumpy appearance, rather like a head of cauliflower. Callus formation is usually due to effects of hormones. While callus formation may be fine for some tissue culture programs, eventually the callus must be stimulated into producing leaves and roots. Try transferring the explant to a new medium with smaller or no concentrations of hormones, or changing the ratios of hormones in the medium.

Having successfully tissue cultured *Dionaea* using flower stalk material, my next project is to try the same with other carnivorous plant species. Is the young, rapidly growing flower stalk tissue from *Sarracenia* relatively free enough of inborn bacterial contaminants as to be viable? Currently I have initiated a number of *Sarracenia* flower stalk cultures that look promising (there is no sign of growth, but very little or no contamination either). As for other species, tissue culture can be tried using information and protocols from additional tissue culture related articles in other issues of Carnivorous Plant Newsletter.

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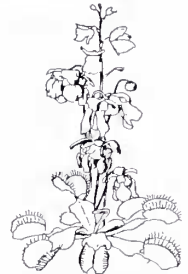
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